



Intraspecific variation in *Eysarcoris aeneus* revealed by geometric morphometrics (Hemiptera: Pentatomidae)

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Abstract: [Aim] *Eysarcoris aeneus*, a main pest of many economic crops, is widely distributed in Palaearctic region with humeri variable. Difference in the humerus length of “rounded humerus form” and “needle-like humerus form” suggests that intraspecific variation may exist in *E. aeneus*. [Methods] In this study, 142 specimens (98 for *E. aeneus* and 44 for the outgroup *E. ventralis*) from 19 regions of China were examined to ascertain the morphological variation of four characters (forewing, hindwing, head and scutellum) between the two forms, “rounded humerus form” and “needle-like humerus form”, respectively, of *E. aeneus* with the use of geometric morphometrics approaches, including multivariate regression, principal component analysis (PCA), canonical variate analysis (CVA) and discriminant function analysis (DFA). [Results] Allometry was not found in all of the four characters (forewing, hindwing, head and scutellum) examined. For the four characters, though the principal component analysis showed overlap between the two forms of *E. aeneus*, the canonical variate analysis showed significant differences between them (P -values for Procrustes distance and Mahalanobis distance were all less than 0.01). The correctly assigned percentages of “rounded humerus form” and “needle-like humerus form” ranged from 67% to 89% specimens based on the four characters using the discriminant function analysis. [Conclusion] This study proved that forewing, hindwing, head and scutellum can serve efficient evaluation criteria in describing intraspecific morphological difference of *E. aeneus*, and scutellum is the most informative character. The geometric morphometric descriptors showed significant shape differences and overlapped centroid size between the two forms of *E. aeneus*, indicating that the analysis of shape is more sensitive for detecting differences among lineages than that of size. Our study shows that geometric morphometrics is efficient in describing intraspecific morphological difference of *E. aeneus*, and lays a foundation for intraspecific variation studies of stink bugs in future.

Key words: *Eysarcoris aeneus*; geometric morphometrics; principal component analysis (PCA); canonical variate analysis (CVA); discriminant function analysis (DFA)

1 INTRODUCTION

Eysarcoris aeneus, belongs to *Eysarcoris* (Hemiptera: Pentatomidae: Pentatominae), is a pest of many economic crops such as wheat, soybean, rice, etc. (Ito, 1978; Learmonth, 1980; Zhang, 1985; Nasiruddin and Roy, 2012). According to some reports, *E. aeneus*, *E. ventralis* and *E. trimaculatus* are main pests of rice, and the rate of pecky rice caused by *E. aeneus* at milk ripe stage was 10.2% (Lee *et al.*, 2009; Nasiruddin and Roy, 2012). *E. aeneus* is a species widely distributed in the Palaearctic region, with humeri

variable. The “needle-like humerus form” was described as a distinct species (*E. parvus*) by Uhler (1896), and then it was treated as a junior synonym of *E. aeneus* by Josifov and Kerzhner (1978). Difference in the humerus length of “needle-like humerus form” and “rounded humerus form” suggests that intraspecific variation may exist in *E. aeneus*.

Geometric morphometrics is a widely used statistical approach in morphology based on Cartesian landmark coordinates (Gilchrist *et al.*, 2000; Debat *et al.*, 2003; Mitteroecker and Gunz, 2009; Bai, 2017). With the use of powerful and comprehensive

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statistical procedures, geometric morphometrics can detect tiny shape differences of a morphological feature (Rohlf and Marcus, 1993; Marcus *et al.*, 2000; Adam *et al.*, 2004). Geometric morphometric analysis has already been proved useful in discriminating sibling species, genus delimitation and even in identification at the individual level among insects (Matias *et al.*, 2001; Baylac *et al.*, 2003; Dujardin *et al.*, 2003; Villemant *et al.*, 2007; Sadeghi *et al.*, 2009; Bai *et al.*, 2014; Su *et al.*, 2015; Giordani *et al.*, 2017).

Eysarcoris can be easily distinguished from other genera because of its basal angle feature in the scutellum with small, yellow, or pale smooth spots. Nevertheless, *Eysarcoris* species show complex variances (Yang, 1962). In our former studies, geometric morphometric approach was employed to distinguish sibling species and the whole genus of *Eysarcoris* in China (Li *et al.*, 2017a, 2017b). The

aim of our present study is to quantify the conspecific morphological features between the two forms of *E. aeneus* based on the four characters (forewing, hindwing, head and scutellum), and to test the possible use of these characters for intraspecific studies of *Eysarcoris*.

2 MATERIALS AND METHODS

2.1 Insects

A total of 142 specimens (41 for “needle-like humerus form” and 57 for “rounded humerus form”) of *E. aeneus* and *E. ventralis* were examined in this study. The number and localities of the specimens were provided in Table 1. *E. ventralis* was chosen as the outgroup (Li *et al.*, 2017b). Samples were collected mainly by manually pick-up or by using sweep nets from fields in China. All specimens were deposited in Taiyuan Normal University and Nankai University in China.

Table 1 Collecting localities and body parts of *Eysarcoris* specimens used in geometric morphometric analysis

Species	Form	Collecting locality	Forewing	Hindwing	Head	Scutellum
<i>E. aeneus</i>	Needle-like humerus form	Linyi, Shandong*	12 ♀ 13 ♂	14 ♀ 13 ♂	20 ♀ 21 ♂	20 ♀ 20 ♂
		Pangquangou Nature Reserve, Shanxi*	8 ♀ 5 ♂	18 ♀ 7 ♂	5 ♀ 5 ♂	4 ♀ 2 ♂
		Luya Mountain Nature Reserve, Shanxi*	1 ♂	8 ♀ 5 ♂	3 ♀ 2 ♂	2 ♀ 2 ♂
		Qingfeng Mountain, Shaanxi*	1 ♀	2 ♀		
	Rounded humerus form	Taibai National Forest Reserve, Shaanxi	3 ♀	3 ♀	3 ♀	3 ♀
		Xunyang, Shaanxi	1 ♀ 1 ♂	1 ♀ 1 ♂	1 ♀ 1 ♂	1 ♀ 1 ♂
		Dongyang, Zhejiang	3 ♀ 1 ♂	3 ♀		
		Huanan, Heilongjiang	4 ♀ 4 ♂	4 ♀ 4 ♂	4 ♀ 4 ♂	4 ♀ 4 ♂
<i>E. ventralis</i>		Beijing*	1 ♀		1 ♀	1 ♀
		Xintai, Shandong	3 ♀	2 ♀	3 ♀	3 ♀
		Linyi, Shandong*	10 ♀ 1 ♂	7 ♀	9 ♀ 2 ♂	9 ♀ 1 ♂
		Luo Mountain, Zhaoyuan, Shandong*	1 ♂		1 ♂	1 ♂
		Tai'an, Shandong*	1 ♂		1 ♂	1 ♂
		Xianren Island, Shandong*	3 ♀ 13 ♂	2 ♀ 3 ♂	3 ♀ 13 ♂	4 ♀ 13 ♂
		Huhuan Forest Park, Changsha, Hunan	1 ♀		1 ♀	1 ♀
		Cangyan Mountain, Hebei*	1 ♀ 2 ♂		1 ♀ 2 ♂	1 ♀ 2 ♂
		Yixian, Hebei*	1 ♂		1 ♂	1 ♂
		Hongtong, Shanxi	1 ♂	1 ♂	1 ♂	1 ♂
		Baxian Mountain, Tianjin*	1 ♀		1 ♀	1 ♀
		Dongyang, Zhejiang	3 ♂	1 ♂	1 ♂	3 ♂
		Houhe Nature Reserve, Hubei*	1 ♀		1 ♀	1 ♀

* The data of specimens of these localities are from Li *et al.* (2017a, 2017b).

2.2 Geometric morphometric approach

For all of the four characters (forewing, hindwing, head and scutellum), the same landmark selection method was used as the former works (Li *et al.*, 2017a, 2017b) (Fig. 1). Landmarks and curves were based on homologous or corresponding criteria. Tps-DIG Ver. 2.05 (Rohlf, 2006) was used to draw curves and landmarks and all semi-

landmarks were converted to landmarks. All pictures and digital procedures were operated by the same person.

Correlations between the Procrustes and tangent shape distances were calculated using tps-SMALL Ver. 1.20 (Rohlf, 2003). Centroid size (CS) was compared to evaluate the overall size variation of the four characters among groups. It was the square root

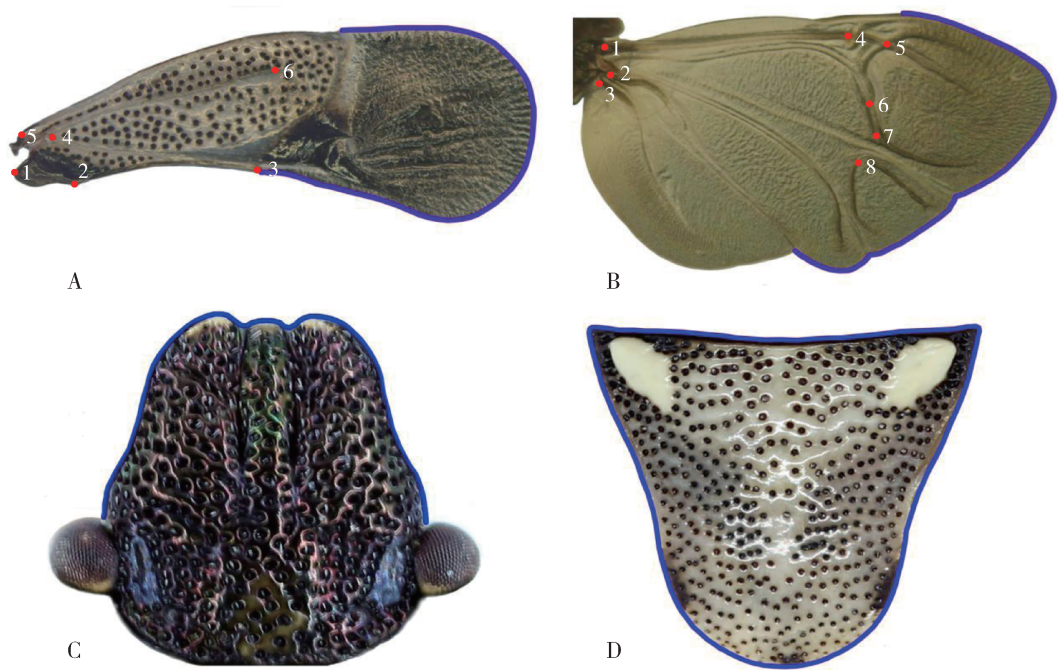


Fig. 1 Landmarks and curves selected
A: Forewing; B: Hindwing; C: Head; D: Scutellum.

of the summed squared distance of each landmark from the center of the form (Zelditch *et al.*, 2004). A one-way ANOVA and HSD pairwise comparisons were conducted using paleontological statistics software Ver. 3. 06 (PAST) (Hammer *et al.*, 2001). To explore how shape varies with size, a multivariate regression of Procrustes coordinates against centroid size was conducted using MorphoJ 1. 06d (Klingenberg, 2011). The significance of the allometry was calculated with a permutation test with 10 000 iterations.

To examine the shape variation of the four characters, the raw landmark data were firstly superimposed using the Procrustes methods in MorphoJ 1. 06d (Bookstein, 1991; Klingenberge, 2011). Shape variations were assessed using a principal component analysis. The relative similarity of the groups was analyzed using canonical variate analysis (CVA). Procrustes distance and Mahalanobis distance were calculated during the CVA analysis. Discriminant function analysis with cross-validation was performed on the Procrustes superimposed coordinates.

3 RESULTS

Variation of the samples in shape space was strongly correlated with tangent space for all the four anatomical characters. For forewing, hindwing, head and scutellum, the correlation between the tangent space regressed onto Procrustes distance were 0. 999265, 0. 999623, 0. 999528 and

0. 999667, respectively.
For forewing and hindwing, the “rounded humerus form” had a bigger centroid size than the “needle-like humerus form”, while for head and scutellum the centroid size of “needle-like humerus form” was bigger than that of the “rounded humerus form” (Fig. 2). For all of the four characters, the difference in centroid size was nonsignificant between the two forms of *E. aeneus* (Table 2). Allometry was not found in all the four characters of the groups since each multivariate regression was statistically nonsignificant ($P > 0.05$).

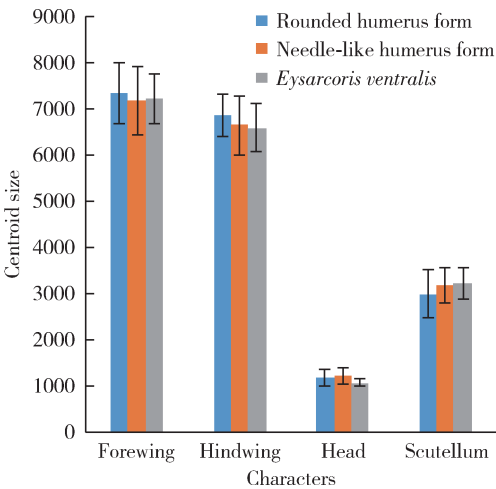


Fig. 2 Comparisons of centroid size of “rounded humerus form” and “needle-like humerus form” of *Eysarcoris aeneus* and *E. ventralis*
Data in the figure are mean $\pm$ SD.

Table 2 Tukey HSD [*P*-values (above) and studentized range statistic *q* (below)] for the centroid size between the “rounded humerus form” and “needle-like humerus form” of *Eysarcoris aeneus* and *E. ventralis*

	Rounded humerus form	Needle-like humerus form	<i>E. ventralis</i>
Forewing			
Rounded humerus form		0.5828	0.7537
Needle-like humerus form	1.4050		0.9591
<i>E. ventralis</i>	1.0150	0.3899	
Hindwing			
Rounded humerus form		0.2676	0.1493
Needle-like humerus form	2.2070		0.9448
<i>E. ventralis</i>	2.6610	0.4544	
Head			
Rounded humerus form		0.4827	0.0168
Needle-like humerus form	1.6280		0.0003
<i>E. ventralis</i>	3.8790	5.5070	
Scutellum			
Rounded humerus form		0.1253	0.0411
Needle-like humerus form	2.7560		0.8848
<i>E. ventralis</i>	3.4220	0.6664	

The shape changes of the different groups were implied by the PCA scatter plots. The first two PCs were plotted to indicate variation along the two axes and accounted for 73.386% , 51.301% , 82.333% and 75.017% of the variation among groups for

forewing, hindwing, head and scutellum, respectively. The principal component analysis reveals overlap between the “rounded humerus form” and “needle-like humerus form” specimens in all the characters (Fig. 3).

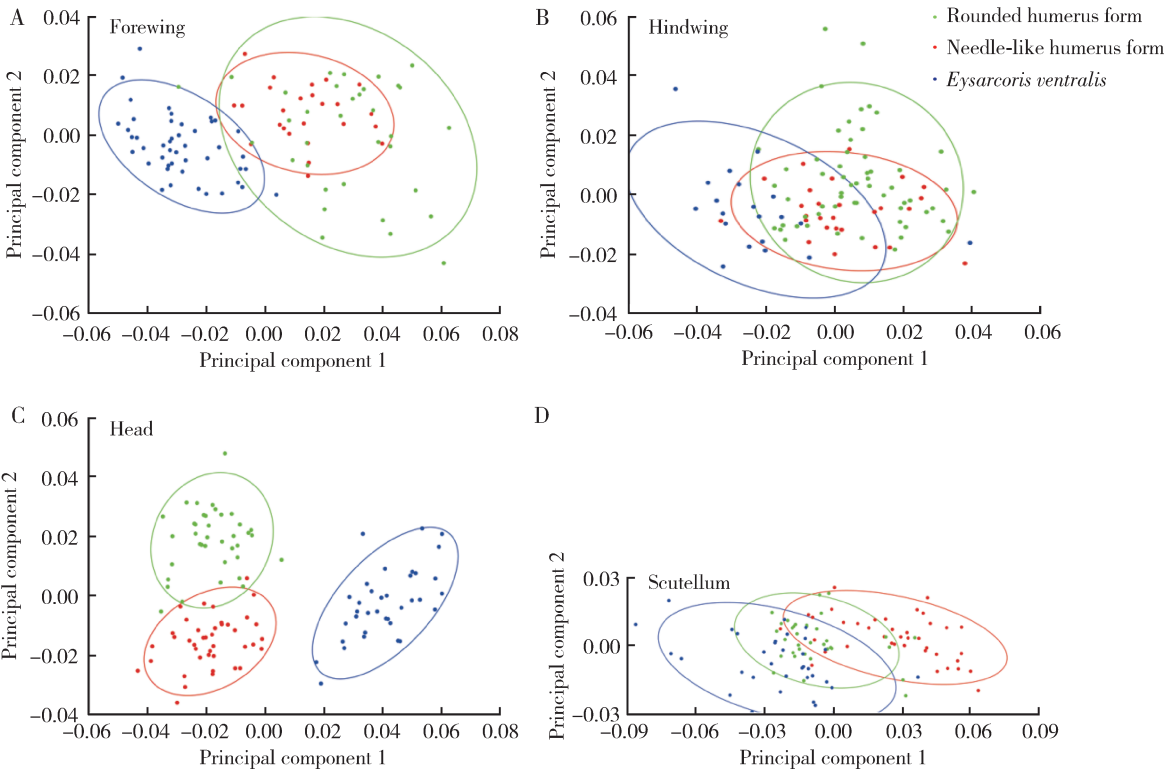


Fig. 3 Principal component analysis (PCA) of “rounded humerus form” and “needle-like humerus form” of *Eysarcoris aeneus* and *E. ventralis*

A: Forewing; B: Hindwing; C: Head; D: Scutellum.

The CVA scatter plots of shape differences all showed that each group occupied different area (Fig. 4). Mahalanobis distances among the three

groups were significantly different ($P < 0.0001$), and Procrustes distances were similar ($P < 0.01$) in all pairwise comparisons (Table 3).

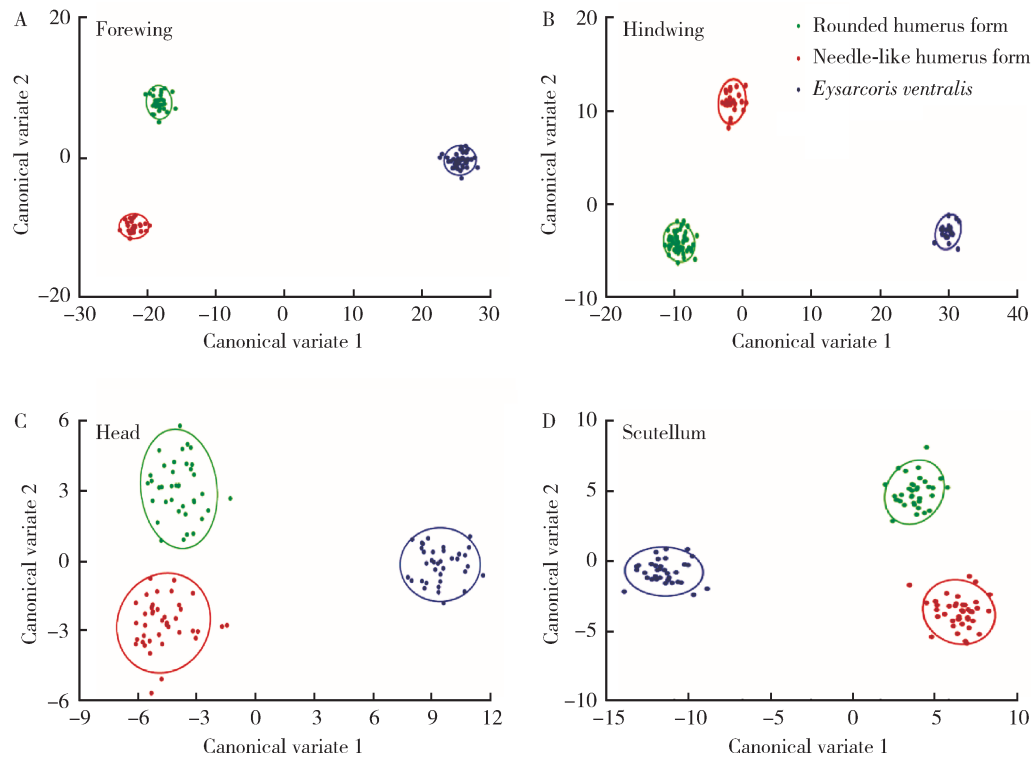


Fig. 4 Canonical variates analysis (CVA) of “rounded humerus form” and “needle-like humerus form” of *Eysarcoris aeneus* and *E. ventralis*
A: Forewing; B: Hindwing; C: Head; D: Scutellum.

Table 3 Difference in Mahalanobis distance and Procrustes distance [P -values (above) , and distance (below)] between the “rounded humerus form” and “needle-like humerus form” of *Eysarcoris aeneus* and *E. ventralis* in the four characters (forewing, hindwing, head and scutellum)

	Mahalanobis distance			Procrustes distance		
	Rounded humerus form	Needle-like humerus form	<i>E. ventralis</i>	Rounded humerus form	Needle-like humerus form	<i>E. ventralis</i>
Forewing						
Rounded humerus form		<0.0001	<0.0001		0.0016	<0.0001
Needle-like humerus form	18.1086		<0.0001	0.0182		<0.0001
<i>E. ventralis</i>	44.6643	48.4641		0.0574	0.0448	
Hindwing						
Rounded humerus form		<0.0001	<0.0001		0.0010	<0.0001
Needle-like humerus form	16.9728		<0.0001	0.0135		<0.0001
<i>E. ventralis</i>	39.2818	34.5077		0.0341	0.0312	
Head						
Rounded humerus form		<0.0001	<0.0001		<0.0001	<0.0001
Needle-like humerus form	5.8227		<0.0001	0.0343		<0.0001
<i>E. ventralis</i>	13.7213	14.3363		0.0627	0.0612	
Scutellum						
Rounded humerus form		<0.0001	<0.0001		<0.0001	<0.0001
Needle-like humerus form	8.9854		<0.0001	0.0359		<0.0001
<i>E. ventralis</i>	16.2703	18.1803		0.0233	0.0532	

Almost all “rounded humerus form” and “needle-like humerus form” specimens were correctly assigned to their own groups, when compared to *E. ventralis*. However, the percentages

correctly assigned of “rounded humerus form” and “needle-like humerus form” specimens from cross-validation were mostly lower than 80% (Table 4).

Table 4 Mahalanobis distances and correct grouping percentage in discriminant function analysis (DFA) (permutation test = 1 000) of “rounded humerus form” (ER) and “needle-like humerus form” (EL) of *Eysarcoris aeneus* and *E. ventralis* (EV)

	Mahalanobis distance			Percentage correctly assigned			
	ER and EL	Compared to EV		ER and EL		Compared to EV	
		ER	EL	ER	EL	ER	EL
Forewing	7. 1493	18. 2040	15. 3005	68%	67%	94%	96%
Hindwing	8. 2789	14. 9685	10. 4564	76%	70%	95%	96%
Head	6. 5737	22. 4490	20. 3487	71%	73%	97%	100%
Scutellum	10. 9763	12. 1191	13. 7435	89%	80%	89%	88%

4 DISCUSSION

Geometric morphometrics is sensitive to small variations and can detect subtle difference within CVA. It was proved to be very powerful in discriminating populations, groups and even subpopulation within the same location (Mendes *et al.*, 2007; Gidaszewski *et al.*, 2009; Oleksa and Tofiski, 2014; Carvajal *et al.*, 2016).

Our morphometric research showed significant differences in forewing, hindwing, head and scutellum between the “rounded humerus form” and “needle-like humerus form” of *E. aeneus*. However, as that has been found for *Anotogaster sieboldii* (Kiyoshi and Hikida, 2012), their centroid sizes do not differ greatly. Hence, an analysis of shape may be more sensitive for detecting differences than that of size, because even when the size overlaps, the lineages differentiate.

The morphometric patterns revealed in our study were discordant to previous molecular studies (Zhao *et al.*, 2015). While there were significant differences in morphology between the “rounded humerus form” and “needle-like humerus form” of *E. aeneus*, the genetic differentiation on the mitochondrial sequence of the two forms was not distinct. Similar results were also found in Large Blue (*Maculinea arion*) (Berezcki *et al.*, 2013). Mahalanobis distance and Procrustes distance between the two forms for all the four characters were smaller than those interspecific ones (Table 3). The DFA analysis gave similar results (Table 4). Shape divergence showed by our study may not reflect specific divergence.

The origins of these differences may be resulted from some adaptive constrains such as the altitude, host plant, temperature, humidity, *etc.* (Gumiel *et*

al., 2003). It suggests that the size changes could be more adaptive than the shape itself (Aytekin *et al.*, 2007; Talloen *et al.*, 2009; Outomuro and Johansson, 2011; Sanzana *et al.*, 2013; Bai *et al.*, 2015). Because of the absence of allometry, and the overlapped centroid size, we supposed that the shape differences we experienced here should not be determined by adaptive factors.

De Queiroz (2007) stated that species are equated with separately evolving metapopulation lineages, and many of the properties, such as morphological, genetic or behavioral differences, are more appropriately interpreted as operational criteria (lines of evidence) relevant to assessing lineage separation. The closer the lineages get to complete differentiation, the easier the diagnosis becomes (Wei *et al.*, 2010; Marchiori *et al.*, 2014). Hence, *E. aeneus* may be in the early stages of speciation.

The two forms of *E. aeneus* can be separated clearly by all of the four characters in CVA with *P*-values for Procrustes distance and Mahalanobis distance all less than 0. 01. However, the correctly assigned percentages of the two forms in DFA were varying (Table 4). This may indicate that the values of the four characters to detect intraspecific difference were not equivalent. The scutellum is the most informative character with almost equal correctly assigned percentages in intra- and interspecific level.

It is a fact that molecular data always tend to receive more emphasis than morphological ones. However, not all of them are suitable for discrimination of all populations or subspecies (Sheppard *et al.*, 1996; Berezcki *et al.*, 2013; Oleksa and Tofiski, 2014; Robins *et al.*, 2014; Hulme-Beaman *et al.*, 2018). Geometric morphometrics have clearly indicated its importance

in clarifying taxonomic and evolutionary relationships among taxa (De la Riva *et al.*, 2001; Gumiel *et al.*, 2003; Campos *et al.*, 2011; Bai *et al.*, 2011, 2012, 2014; Dehon *et al.*, 2014), and it was proved to be more useful than molecular ones in some studies (Bloch *et al.*, 2010; Bereczki *et al.*, 2013; Oleksa and Tofiski, 2014). In the present work, we are able to show that geometric morphometrics is efficient in describing intraspecific shape difference of *E. aeneus*.

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基于几何形态学的北二星蝽种内形态差异分析

(半翅目: 蝽科)

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摘要:【目的】北二星蝽 *Eysarcoris aeneus* 是一种广泛分布于古北区的重要农业害虫,可危害多种经济作物。其刺肩型和钝肩型前胸背板后侧角长度存在差异,暗示其种内变异的存在。【方法】基于几何形态学的多元回归分析(multivariate regression)、主成分分析(principal component analysis, PCA)、典型变量分析(canonical variate analysis, CVA)和判别函数分析(discriminant function analysis, DFA),对采集于中国 19 个地区的 142 头标本(98 头北二星蝽标本和 44 头作为外群的广二星蝽 *E. ventralis* 标本)的前翅、后翅、头和小盾片 4 个性状进行分析比较,研究北二星蝽钝肩型和刺肩型之间的形态变异。【结果】对于所研究的 4 个性状来说,北二星蝽钝肩型和刺肩型标本间均未检测到异速生长的存在。主成分分析中,钝肩型和刺肩型的标本均有重叠,典型变量分析则显示它们之间存在显著差异(马氏距离和普氏距离的 P 值均小于 0.01)。判别函数分析显示,依据这 4 个性状两型样本间的正确判别率在 67% ~ 89% 之间。【结论】结果表明,前翅、后翅、头和小盾片都可以作为北二星蝽钝肩型和刺肩型之间形态变异的评价指标,小盾片特征具有最高水平的鉴别价值;对于所研究的这 4 个性状来说,北二星蝽钝肩型和刺肩型之间都表现出显著的形状差异,而它们的质心距离差异不显著,说明相对于体型大小差异分析,形状差异分析能更灵敏地揭示谱系间的变异。本研究表明几何形态学可以有效地描述北二星蝽的种内形态变异,为蝽类昆虫种内变异的研究奠定了基础。

关键词: 北二星蝽; 几何形态学; 主成分分析; 典型变量分析; 判别函数分析

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